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PRE-APPEAL BRIEF REQUEST FOR REVIEW		
	Attorney Docket No.	RICE-050
	Confirmation No.	3065
	First Named Inventor	MACKAY, CHARLES REAY
	Application Number	10/584,480
	Filing Date	April 17, 2007
	Group Art Unit	1632
	Examiner Name	WILSON, MICHAEL C.
	Title: "Transgenic Mouse Comprising a Polynucleotide Encoding Human or Humanized C5aR and Methods of Production and Use"	

Applicant requests review of the final rejection in the above-identified application.
No amendments are being filed with this request.

This request is being filed with a notice of appeal.

The review is requested for the reason(s) stated on the attached sheet(s).
Note: No more than five (5) pages may be provided

I am the

☐ applicant/inventor

☐ assignee of record of the entire interest

☒ attorney or agent of record
Registration number

☐ attorney or agent acting under 37 C.F.R. 1.34

/Carol L. Francis, Reg. No. 36,513/
Signature

Carol L. Francis, Ph.D.
Reg. No. 36,513

May 16, 2011
Date

NOTE: Signatures of all the inventors or assignees of record of the entire interest or their representative(s) are required.

Remarks

Claims 1-5, 10, 14-20, 22, 27-28, 30-35, and 40 are currently pending. The claimed invention relates to transgenic mice that are homozygous for a human or humanized C5a receptor ("C5aR"), wherein the endogenous C5aR coding sequences are disrupted and wherein C5a endogenous to the mouse binds to and effects signalling of the human or humanized C5aR (referred to herein for brevity as the "transgenic human C5aR mouse"). The claimed invention also relates to cells isolated from such transgenic mice, as well as methods of use.

C5a is a product of proteolysis of complement proteins triggered in response to inflammation or the formation of antigen/antibody complexes (immune complexes) (specification, pg. 1, lines 13-33). Once produced, C5a binds C5aR, which in turn mediates development of inflammation and conditions such as arthritis that can result from such inflammatory responses. In the conventional mouse inflammation model, an inflammatory response is induced in healthy, wild-type mice by administering sera from arthritic K/BxN mice (referred to as "arthritis-inducing sera"), that triggers an inflammatory response that results in production of C5a by proteolysis from complement protein, binding of C5a to C5aR, and development of arthritis in mice (specification at pg. 60, lines 5-8). The role of C5a and C5aR in mediating inflammation-induced arthritis is confirmed by the observation that mice that lack C5aR do not develop inflammation in this model (specification at pg. 40, line 28-30).

The claimed transgenic human C5aR have endogenous mouse complement proteins; thus, administration of arthritis-inducing sera causes production of mouse C5a. The claimed transgenic human C5aR mice have disrupted endogenous C5aR coding sequences (see, e.g., claim 1), and thus lack functional endogenous mouse C5aR. Any C5aR-mediated effect is thus due to binding of *endogenous mouse C5a to the human or humanized C5aR*. The specification provides ample evidence that arthritis development in the transgenic human C5aR mice is due to binding of endogenous mouse C5a ligand to human C5aR (specification pg. 62, line 20 to pg. 63 line 4): 1) Mice transgenic for human C5aR and having endogenous mouse C5a ligand, develop arthritis following administration of arthritis-inducing sera *just as do nontransgenic control mice*; and 2) An antibody specific for human C5aR blocks induction of arthritis in transgenic human C5aR mice, *but not in nontransgenic control mice*. These data show that endogenous mouse C5a binding to human C5aR in the transgenic human C5aR mice mediates development of arthritis following induction of inflammation by arthritis-inducing sera.

All pending claims stand rejected under §103(a) as being unpatentable over Sato (Thrombosis and Haemostasis (1999) 82(2):865-869), Roebroek (Methods in Molecular Biology (2003) 209:187-200), Homanics (Methods in Alcohol Related Neuroscience Research (2002), pg. 31-61), Lester et al, (Curr. Opin. Drug Discov. and Dev. (2003) 6(5):663-639), Champtiaux (Curr. Drug Targets, CNS & Neuro. Dis.(2002)1:319-330), Girardi (J. Clin. Invest. (2003) 112(11):1644-1654) in view of Burmer (WO 02/61087) and Cain (Biochemical Pharm., 2001 61:1571-1579) and as supported by Drago (Cell. Mol. Life Sci 2003 60:1267-1280), Gu (Dev. Cell 2003 5:45-57), Belmont (WO 2002/059262) and Kane (WO 2003/027252). Office Action dated February 16, 2011. In summary:

- **Sato** is cited for its alleged disclosure of a knock-in mouse in which an endogenous gene is replaced with an exogenous gene or a mutant form of the endogenous gene.
- **Roebroek** is cited for its alleged disclosure of various strategies for making knock-in mice, including its alleged disclosure of disruption of an endogenous mouse gene and replacement with a human receptor homolog, where the human receptor binds the endogenous ligand and functions in vivo. (None are receptors for complement proteins, their proteolytic products, or anaphylatoxins).
- **Homanics** is cited for its alleged disclosure of a transgenic mouse having an endogenous receptor disrupted and replaced with a human receptor homolog. (This reference only describes $GABA_A$ receptor knockin mice and the effects of benzodiazepines (2.5.4)).
- **Lester** and **Champtiaux** are cited for alleged disclosure of receptor knock-in mice. (Each of these references relate to nicotinic receptors.)
- **Girardi** is cited for its alleged disclosure of "knocking out" a mouse C5aR gene.
- **Burmer** is cited for its alleged disclosure of human C5aR cDNA.
- **Cain** is cited for its alleged disclosure of mutated human C5aR that functioned in rat cells.
- **Drago**, **Gu**, **Belmont** and **Kane** are cited for their alleged disclosure of a humanized receptor that bound a mouse ligand. (None of these references relate to receptors for complement proteins, their proteolytic products, or anaphylatoxins)

Of all the references cited, only Girardi, Burmer, and Cain are in the relevant field. The remaining references relate to either general methods or to alleged examples of humanized receptors. None relate to receptors for anaphylatoxins such as complement proteins or their proteolytic products.

Applicants submit that the Examiner has failed to establish a *prima facie* case of obviousness, and has committed several clear errors in his analysis of the evidence, the characterization of the art, in construing the language of the claims, and in characterizing the evidence in the application in support of the claimed invention.

Applicants submit the Examiner has committed clear error in failing to consider the full weight of the evidence. Not only have Applicants submitted strong evidence of whether the ordinarily skilled artisan would have a reasonable expectation of success in the context of the claimed invention, but in addition, the Examiner has erred in his characterization of his evidence. The Examiner has failed to give the evidence full weight, thus committing further clear error.

Applicants argued throughout prosecution that the claimed invention is not obvious because there was no reasonable expectation of success that a mouse transgenic for a human or humanized C5aR would bind endogenous mouse C5a and thus provide a model of inflammation, e.g., inflammatory-induced arthritis. Applicants submitted evidentiary support, including a Declaration of Craig Gerard, MD, PhD under 37 CFR §1.132 ("Gerard Declaration"), that the ordinarily skilled artisan would have no reasonable expectation of success in this regard, even in view of the cited art, based on knowledge of C5aR and related receptors. Gerard Declaration, par. 12; see also pars. 13-16. At par. 14, the Gerard Declaration describes the amino acid sequence divergence of mouse and human C5a and C5aRs, and concludes that in view of this sequence diversity a "Skilled Person" would not have predicted that mouse

C5a would bind human C5aR. At par. 16, the Gerard Declaration states that cross species functions of anaphylatoxin ligands and their receptor receptors were well known to be unpredictable, and provides a specific example relating to interaction of human C4a with C3a receptors in guinea pigs but not with human C3a receptors. In support of these statements, the Gerard Declaration cited the Woodruff et al.,[1] Hugli et al.[2], and Lienenklaus et al.,[3] publications. Despite this showing, the Examiner persists in his opinion that it was reasonably predictable that mouse c5a would bind human C5aR

The Examiner persists in his position based on examples of human receptors that allegedly bind mouse ligands. In rebuttal, Applicants pointed to still further references as evidence that, due to the diversity of cross-species ligand-receptor interactions, the ordinarily skilled artisan can not *a priori* predict binding of a ligand of one species to its corresponding receptor in another species. These references, which were also cited in an Information Disclosure Statement (IDS)), included:

- Layton *et al.*, 1994, J Biol. Chem. 269(25):17048-17055 shows that human LIF can bind mouse LIF-receptor(LIF-R), but that mouse LIF does not bind human LIF-R.
- Mosmann *et al.*, 1987, J. Immunol. 138:1813-1816 shows that mouse IL-4 does not bind human IL-4R, and that human IL-4 does not bind mouse IL-4R.
- Liu *et al.*, 1996, Cytokine 8(8):613-621 shows that human IL-2 does bind both mouse and human IL-2R equally, that mouse IL-2 binds mouse IL-2R, but mouse IL-2 does not bind human IL-2R very well.
- Smith *et al.*, 1986, J. Biol. Chem. 261(32):14871-14874, shows that mouse and human TNF bind human TNF-receptor (TNF-R) with similar affinities but that human TNF binds to mouse TNF-R with considerably lower affinity than mouse TNF binds to mouse TNF-R. This relationship is opposite to that observed for LIF and IL-2.

The Examiner has failed to consider this further evidence.

The Examiner has also erred in characterization of at least three of the references upon which the §103 rejection is based. First, Gu, as previously argued, does not teach a human or humanized receptor. Figure 1A of Gu shows the structure of the Npn-1 gene, the sequence of the wildtype (mouse) gene in the CUB domain (green oval) and the 7 amino acid substitutions made in the gene to create the Npn-1Sema-gene. The mutations were made in the rat Npn-1 gene. The wildtype sequence shown in Figure 1A of Gu matches that of mouse and rat Npn-1. It does not match human Npn-1. The 7 amino acid substitutions made do not change the sequence to that of human Npn-1. Instead, they changed charged residues to one of opposite charge (e.g. His to Glu, Glu to Arg, Lys to Asp, Asp to Lys, Arg to Glu) and changed uncharged Ser residues to Ala. Figure 1B of Gu et al shows how a targeting vector containing the mutant gene (Npn-1Sema-) was introduced into the mouse by homologous recombination to replace the wildtype gene and create the knockin mouse. The word "human" does not appear anywhere in the text of Gu et al. There is no indication in this paper that the mutant gene is a humanized version of mouse Npn-1.

The Examiner also erred in characterization of Drago. The Examiner asserts that Drago describes a knock-in mouse with a humanized receptor known to bind the mouse ligand, and points to the

[1] Woodruff et al. (2001) Inflamm. 25:171-177, first submitted with response filed Nov. 16, 2009.
[2] Hugli et al. (1983) Mol. Immunol. 20:637:64, first submitted with response filed Nov. 16, 2009.
[3] Lienenklaus et al. (1998) J. Immunol. 161:2089-2093, first submitted with response filed Nov. 16, 2009.

L9S' knockin with a Leu-to-Ser point mutation as an example of a humanized receptor that binds mouse ligand. This is wrong. The L9S' mutation was introduced into mice. The Leu to Ser mutation was made in the sequence: VTLCISVLLSLTVFLLLIT (L in bold was change to Ser). This sequence is identical in the mouse and rat alpha-4 nAChR subunit. The human sequence in this region is ITLCISVLLSLTVFLLLIT, i.e. only the first amino acid is different. The Leu to Ser change does not introduce a human amino acid, thus the sequence was not "humanized.

The Examiner also committed clear error in characterization of the Cain reference. The Examiner points to pg. 1573, col.2, §3.2 to support his assertion that "Cain taught mutated human C5aR functioned in rat cells. Office Action pg 8, first par. Cain's statement that the mutated human C5aR "functioned in rat cells" refers to expression of the mutated human C5aR in RBL-2H3 cells, which are a rat cell line. There is no rat C5a present in this assay – rather human C5a was used and it might be expected that human C5a would bind to human C5aR even when the human C5aR is expressed in rat cells. Thus the Examiner errs in relying on this mischaracterization of Cain to conclude that "despite the lack of 100% homology of mouse and human C5aR, those of ordinary skill would have known how to make a mutated human C5aR that functioned in murine cells and had a reasonable expectation of mouse C5a binding human C4aR as evidenced by Cain." Office Action, pg. 7, first paragraph. The Examiner's logic is faulty and based on an erroneous reading of Cain.

Second, Cain is directed to binding of a synthetic ligand to a "mouse-cized" version of a human C5aR. Cain's sole focus is to explore why a synthetic drug found to bind a mouse C5aR did not have the same effect on human C5aR. As stated in paragraphs 18-19 of the Gerard Declaration, Cain is evidence as to the problem of interspecies variability of binding of ligands to C5aRs of different animals. Indeed, the Cain reference admits to this interspecies variability. On pg. 1572 column 1, last full paragraph, Cain refers to the "complicated patterns of affinities and responsiveness to C5a" and states that differences in affinities "are due to species-specific variations in the primary structure of C5aRs". Cain, thus, is evidence that supports Applicants position that the ordinarily skilled artisan had no reasonable expectation of success in making the claimed transgenic mouse. In addition, the claims here are directed to a "humanized" C5aR – and Cain simply provides no teaching of how to humanize a non-human C5aR or that such would be expected to bind mouse C5a ligand as asserted by the Examiner.

The Examiner's rebuttal of Applicants arguments simply asserts that variation in binding of agonists to mouse and human C5aR fails to indicate mouse C5a will not bind and effect signaling of human C5aR. Office Action pg. 8, bottom. This is in direct contradiction to the evidence provided in the Gerard Declaration, see, e.g., par. 19. The Examiner has failed to submit evidence of similar weight in support of this otherwise unsupported conclusion based on his reading of Cain.

The Examiner has also committed clear error in ignoring the language of the independent claims, and fails to take into consideration language of dependent claims. The Examiner states that "the claims are not limited to a mouse expressing the entire human C5aR in the absence of the mouse C5aR." See, e.g., Office Action, pg. 6 last full paragraph; see also text bridging pgs. 6-7. Claim 1 requires the endogenous C5aR coding sequences are disrupted, and thus require no functional mouse C5aR. Further dependent claims, such as claims 2, 3, 16 and 18, recite a human C5aR.

The Examiner also commits a second clear error in construing the claims. In the Office Action at pg. 8, second full paragraph he states "The claims are not limited to obtaining binding and proper signalling of mouse C5a and human C5aR." To the contrary, claim 1 recites "wherein the C5a endogenous to the mouse binds to and effects signalling of the human or humanized C5aR".

Finally, the Examiner has committed clear error in characterization of the evidence in the specification supporting the claimed invention. The Examiner states that "it is unclear mouse C5a does bind to and effect proper signaling of the human C5aR. Accordingly it is not readily apparent that binding of proper signaling of mouse C5a and human C5aR can be considered the 'unexpected results'." (Office Action pgs. 7-8, emphasis in original). This statement is clear error. The transgenic mice described and claimed **do**, in fact have a "normal" phenotype. When inflammation and production of C5a is induced by administration of the arthritis-inducing antisera, the transgenic mice developed arthritis symptoms, *similar to those observed in a normal, non-transgenic mouse*. Moreover, an antibody specific for human C5aR *blocked* induction of arthritis in transgenic human C5aR mice, *but not in nontransgenic control mice*. Thus the human C5aR binding to the endogenous mouse C5a mediated the arthritis symptoms in the claimed transgenic mice. There is no other reasonable technical conclusion to be drawn from this data.

Conclusion

Applicants have provided ample arguments, supported by evidence, e.g., in the form of the Gerard Declaration, that within the relevant field, it was not predictable that mouse C5a would bind a human or humanized C5aR and further would effect proper signaling, as evidenced by the development of arthritis symptoms in transgenic human C5aR mice following administration of antisera that induces inflammation and mouse C5a production. Applicants submit that the facts set forth above demonstrate a clear deficiency in the *prima facie* case made by the Examiner to support a rejection under 35 U.S.C. §103. The Examiner has committed clear error in characterization of the claims and the art, failed to take into consideration dependent claims, and further failed to give full evidentiary weight to the Gerard Declaration.

The Examiner's rejection of claims 1-5, 10, 14-20, 22, 27-28, 30-35, and 40 over the combined disclosures of the cited references cannot be properly maintained. Reversal of the rejection is respectfully requested.

The Commissioner is hereby authorized to charge any underpayment of fees associated with this communication, including any necessary fees for extensions of time, or credit any overpayment to Deposit Account No. 50-0815, order number RICE-050.

Respectfully submitted,

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Date: May 16, 2011

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